REMARKS

Amendments to the Claims

The Applicants respectfully ask the Examiner to replace all prior versions and listings of claims in the present application with the listing of claims currently provided. Claims 4-8, 45-53, 55, 57-64, 96-122, 126-146, 149-168 and 170-178 were amended, Claims 147, 148 and 169 were canceled, and claims 179-206 are new.

Amendment support for Claims 4-8 can be found throughout the present specification. Support for an acceptor being a fluorophore can be found, *e.g.*, at pg. 71, ¶ 3 through pg. 72, ¶ 1; pg. 89, ¶ 1; original Claims 50, 51, 64-67 and 94; and Table 6. Support for a BoNT/A recognition sequence comprising SEQ ID NO: 27 or SEQ ID NO: 29 can be found, *e.g.*, at pg. 40, ¶ 3 through pg. 41, ¶ 1.

Amendment support for Claims 52 and 58-63, 115-117, 184-195 and 200-211 can be found throughout the present specification. Support for the specified donor fluorophores and acceptor fluorophores can be found, *e.g.*, at pg. 83, ¶ 1; pg. 85, ¶ 2 through pg. 86, ¶ 1; original Claims 56; and Table 6. Support for the specified non-fluorescent acceptors can be found, *e.g.*, at pg. 90, ¶ 1. Support for the specified fluorescent proteins can be found, *e.g.*, at pg. 88, ¶ 2.

Amendment support for Claims 102-104 can be found throughout the present specification. Support for the donor fluorophore, the acceptor or both the donor fluorophore and acceptor not positioned with amino acids 191 to 202 can be found, *e.g.*, at pg. 90, ¶ 1; pg. 93, ¶ 2 through pg. 94, ¶ 1; and Example 1. Support for a BoNT/A recognition sequence comprising SEQ ID NO: 29 or SEQ ID NO: 30 can be found, *e.g.*, at pg. 40, ¶ 3 through pg. 41, ¶ 1.

Amendment support for Claims 126 and 133, 198, 199, 203-208, 210 and 211can be found throughout the present specification. Support for a BoNT/A recognition sequence comprising SEQ ID NO: 27 SEQ ID NO: 29, 137 to 206 of SEQ ID NO: 2 and 134 to 206 0f SEQ ID NO: 2 and SEQ ID NO: 2 can be found, *e.g.*, at pg. 24, ¶ 3 through pg. 25, ¶ 1; and

pg. 40, ¶ 3 through pg. 42, ¶ 1. Support for BFP as a donor fluorophore and BFP as an acceptor fluorophore can be found, *e.g.*, at pg. 88, ¶ 2.

Amendment support for Claims 96-101, 118-121, 196 and 197 can be found throughout the present specification. For example, support for a substrate having at most 20 residues, at most 40 residues, at most 50 residues, at most 100 residues, at most 150 residues and at most 200 residues can be found, *e.g.*, at pg. 11, ¶ 3; pg. 21, ¶ 1; and original Claims 61-63.

Amendment support for Claims 122 and 146, 149-152 can be found throughout the present specification. For example, support for a donor fluorophore and an acceptor being separated by at most six residues, at most eight residues, at most ten residues, at most fifteen residues at most twenty residues, at most twenty five residues, at most thirty residues, at most thirty five residues or at most forty residues can be found, *e.g.*, at pg. 11, ¶ 3; and pg. 21, ¶ 1.

Amendment support for Claims 52, 55, 58-61, 184-187 190-193, 210 and 211 can be found throughout the present specification. Amendment support for chemical names of trademark BODIPY fluorescent dyes can be found, *e.g.*, at pg. 83, ¶ 1. The chemical names for the trademark Alexa Fluor fluorescent dyes are proprietary information that the company Invitrogen, Inc. (Carlsbad, CA) will not release to the public. Support for these dyes are given in terms of emission maxima for donor fluorophores and excitation maxima for acceptor fluorophores and can be found in Haugland, *Handbook of Fluorescent Probes and Research Chemicals* 6th Edition, Molecular Probes, Inc., Eugene, Oregon, 1996, which was incorporated by reference, see, *e.g.*, pg. 72, ¶ 1.

Rejection Pursuant to 35 U.S.C. § 112, ¶ 1 Written Description

The Examiner has rejected Claims 4-8, 45-53, 55, 57-64, 96-101, 142-144, 149-154, 160-164 and 169-175 as allegedly being indefinite under 35 U.S.C. § 112, ¶ 1 indicating that the claims contain subject matter that was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventors had possession of the claimed invention at the time the application was filed. Specifically, the Examiner contends

that there is no written description support for a donor and acceptor being separated by at least 14, at least 50 residues, at least 75 residues, at least 100 residues, at least 125 residues, at least 150 residues or at least 200 residues, The Examiner also contends that there is no written description support for a peptide or peptidomimetic having at most 500 residues, at most 600 residues, at most 700 residues; or at least 300 residues, at least 400 residues, at least 500 residues, at least 600 residues or at least 700 residues. The Applicants respectfully ask for reconsideration under 37 C.F.R. § 1.111.

According to MPEP 2163.02, the fundamental factual inquiry is whether the specification conveys with reasonable clarity to those skilled in the art that, as of the filing date sought, applicant was in possession of the invention as now claimed. The subject matter of the claim need not be described literally (*i.e.*, using the same terms or *in haec verba*) in order for the disclosure to satisfy the description requirement.

The Applicants respectfully submit that there is adequate written disclosure throughout the present specification supporting a BoNT/A substrate from at least 300 residues, at least 400 residues, at least 500 residues, at least 600 residues or at least 700 residues, as well as, for BoNT/A substrates from at most 500 residues, at most 600 residues or at most 700 residues. Additionally, there is adequate written disclosure throughout the present specification supporting a donor fluorophore and acceptor separated by at least 14 residues, at least 50 residues, at least 75 residues, at least 100 residues, at least 125 residues, at least 150 residues and at least 200 residues.

A BoNT/A substrate comprises in part, a donor fluorophore, and acceptor and a BoNT/A recognition site. First, the present specification has, in part, written description support for embodiments in which a BoNT/A substrate comprises at least 13 residues to at least 206 residues and at most 13 residues to at most 206 residues. For example, recognition sequence lengths of 13, 15, 16, 17, 18, 70 and 73 as disclosed, *e.g.*, at pg. 37, ¶ 3 indicate a BoNT/A substrate of at least or at most these lengths. Further disclosure indicates that a BoNT/A recognition sequence can comprise a S1, a S2, a S3 and/or a S4 α-helical motif, *e.g.*, at pg. 25, ¶ 3; pg. 30, ¶ 1 through pg. 31, ¶ 1; pg. 37, ¶ 2 through pg. 44, ¶ 1 and FIG. 4A. Thus, a BoNT/A recognition sequence comprising a S1 α-helical motif can have a

length, such as, *e.g.*, 180-206 amino acids; a BoNT/A recognition sequence comprising a S2 α-helical motif can have a length, such as, *e.g.*, 166-206 amino acids; a BoNT/A recognition sequence comprising a S3 α-helical motif can have a length, such as, *e.g.*, 152-206 amino acids; and a BoNT/A recognition sequence comprising a S4 α-helical motif can have a length, such as, *e.g.*, 56-206 amino acids. Again, since a BoNT/A substrate comprises a BoNT/A recognition sequence, a BoNT/A substrate must be of at least these lengths or at most these lengths. Thus, at a minimum, this disclosure supports 1) certain embodiments where a BoNT/A substrate length can be from at least 13 residues to at least 206 residues because one of the substrates components, the BoNT/A recognition sequence can be from at least 13 residues to at least 206 residues; and 2) certain embodiments where a BoNT/A substrate length can be from at most 13 residues to at most 206 residues because one of the substrates components, the BoNT/A recognition sequence can be from at most 13 residues to at most 206 residues.

Second, the present specification has written description support for other embodiments in which a BoNT/A substrate comprises at least 13 residues to at least 682 residues and at most 13 residues to at most 682 residues. For example, the location of a donor fluorophore or an acceptor can be located at either the amino-terminus or the carboxyl-terminus, see, *e.g.*, at pg. 90, ¶ 2. A donor fluorophore or an acceptor can be a molecule chemically linked to the end of the BoNT/A recognition sequence, see, *e.g.*, Example 1. Thus, combining the BoNT/A recognition sequences of at least 13 residues to at least 206 residues discussed above, with terminally-linked donor fluorophore or an acceptor will result in a BoNT/A substrate that can be at least 15 residues to at least 208 residues, with the donor fluorophore and acceptor separated by at least 13 residues to at least 206 residues. Likewise, a donor fluorophore and an acceptor can be internally placed within the BoNT/A recognition sequence, see, *e.g.*, pg. 28, ¶ 2; and pg. 46, ¶ 1. Thus, combining the BoNT/A recognition sequences of at most 13 to at most 206 residues discussed above, with terminally-linked donor fluorophore or an acceptor will result in a BoNT/A substrate that can be at most 13 residues to at most 206 residues when the , with the donor fluorophore and acceptor separated by at most six residues to at most 206 residues.

Furthermore, a donor fluorophore or an acceptor can also be a genetically encoded fluorescent proteins can be found, *e.g.*, at pg. 88, ¶ 2. A fluorescent protein, like GFP, BFP, CFP, YFP and RFP, is typically 238 amino acids in length. Thus, combining the BoNT/A recognition sequences of at least 13 residues to at least 206 residues discussed above, with terminally-linked donor and acceptor fluorescent proteins will result in a BoNT/A substrate that can be from at least residues 489 to at least 682 residues, with the donor and acceptor fluorescent proteins separated by at least 13 residues to at least 206 residues.

Third, a BoNT/A substrate can also comprises, in part, additional components, such as, *e.g.*, flexible spacer sequence, an affinity tag, an epitope tag, an immunoglobulin hinge region, an N-hydroxysuccinimide linker, a peptide or peptidomimetic hairpin turn, or a hydrophilic sequence, see, *e.g.*, pg. 94, ¶ 3 through pg. 95, ¶ 1. A BoNT/A substrate comprising one copy of a five amino acid flexible spacer sequence, a six amino acid His affinity tag and a ten amino acid c-myc epitope, combined with a BoNT/A recognition sequence and two genetically-encoded fluorescent proteins from at least 489 to at least 682 residues discussed above, would result in a BoNT/A substrate of at least 510 to at least 703 residues.

Thus, the Applicants respectfully submit that there is adequate written description support for the substrate lengths claimed because specification conveys with reasonable clarity to those skilled in the art that, as of the filing date sought, applicants were in possession of the invention as now claimed. Therefore, the Applicants respectfully request withdrawal of the 35 U.S.C. § 112, ¶ 1 written description rejection for Claims 4-8, 45-53, 55, 57-64, 96-101, 142-144, 149-154, 160-164 and 169-175.

Rejection Pursuant to 35 U.S.C. §102 (e) Anticipation

The Examiner has rejected Claims 102-112 and 118-122 as allegedly anticipated under 35 U.S.C. §102(e) by James J. Schmidt and Robert G. Stafford, *High Throughput Assays for the Proteolytic Activities of Clostridium Neurotoxins*, U.S. Patent 6,762,280 (priority filing date Sep. 25, 2000), hereafter the "Schmidt patent.". Specifically, the Examiner contends that the peptides SEQ ID NO: 11 and SEQ ID NO: 12 disclosed in the Schmidt patent anticipate the peptides set forth in Claims 102-104. The Applicants respectfully ask for reconsideration under 37 C.F.R. § 1.111.

According to MPEP 21331, for a reference to anticipated a pending claim, that reference must either expressly or inherently teach each and every element of the pending claim.

The BoNT/A substrate of Claim 102 and the claims depending from this independent claim recite, in part, a BoNT/A substrate comprising "a donor fluorophore" and "an acceptor." The peptide substrates SEQ ID NO: 11 and SEQ ID NO: 12 only have one fluorophore located at position 1 of both substrates. Thus, the peptides of SEQ ID NO: 11 and SEQ ID NO: 12 of the Schmidt patent do not read on Claims 102-112 and 118-122.

Therefore, the Applicants respectfully submit that the pending claims are not anticipated by the Schmidt patent and respectfully request withdrawal of the 35 U.S.C. §102(e) anticipation rejection for Claims 102-112 and 118-122.

I. Obviousness rejections over Schmidt in view of Holskin

The Examiner has rejected Claims 102 and 113-117 as allegedly being obvious under 35 U.S.C. § 103(a) over Schmidt patent in view of B. P. Holskin et al., *A Continuous Fluorescence-based Assay of Human Cytomegalovirus Protease Using a Peptide Substrate*, 226 (1) Anal. Biochem. 148-155 (1995), hereafter the "Holskin reference."

The Examiner contends that it would have been *prima facie* obvious to a person of ordinary skill in the art at the time the invention was made to combine the teaching of these references and come up with the BoNT/A substrates as presently claimed in Claims 102 and 113-117. Specifically, the Examiner argues that it would have been obvious to modify the peptide substrates of SEQ ID NO: 11 and SEQ ID NO: 12 disclosed in the Schmidt patent with the fluorophore EDANS and the quencher DABCYL disclosed in the Holskin reference. The Applicants respectfully ask for reconsideration under 37 C.F.R. § 1.111.

Schmidt and Holskin teach away from the claimed invention.

According to MPEP §2143.03, to establish *prima facie* obviousness of a claimed invention, all the claim limitations must be taught or suggested by the prior art. The Applicants

respectfully submit that it would not be obvious to combine the cited references because the Schmidt patent and the Holskin reference teach away from a Clostridial toxin substrate having either a donor or quencher not present in the amino acid segment 191 to 202 of SEQ ID NO: 2.

The BoNT/A substrate of Claim 102 and the claims depending from this independent claim recite, in part, "wherein said donor fluorophore, said acceptor, or both said donor fluorophore and said acceptor is not positioned within amino acids 191 to 202 of SEQ ID NO: 2, or a peptidomimetic thereof." However, the Schmidt patent teaches that the donor and quencher of a type I substrate can only be separated by 2-5 amino acids and that the placement of both the donor and quencher can only be located at certain positions within amino acids 191 to 202 of SEQ ID NO: 2 and, thus, teaches away from the claimed invention which is directed towards FRET substrates comprising a donor fluorophore and an acceptor located outside of amino acids 191 to 202 of SEQ ID NO: 2. Additionally, the Schmidt patent teaches that a type II substrates of SEQ ID NO: 11 and SEQ ID NO: 12 contain only one fluorophore and, thus, teaches away from the claimed invention which is directed towards FRET substrates comprising a donor fluorphore and an acceptor.

Schmidt type I substrates

The Schmidt patent discloses two types of substrates, see abstract. Type I peptide substrates for BoNT/A, BoNT/B, BoNT/ D and BoNT/F useful for quench release assays, see, *e.g.*, col. 5, line 8 through col. 6, line 53. The Schmidt patent teaches that the type I substrates are used in a radiating (contact) quenching assay, see, *e.g.*, col. 4, lines 14-17; col. 5, lines 9-15; and col. 6, lines 54-59. That the peptide substrates disclosed in the Schmidt patent rely on radiating quenching is evident through the design of the peptide substrates.

First, close proximity of the donor and quencher is essential to achieve radiating quenching because this type of quenching relies on the physical interaction of the electron orbits of the donor and the quencher, see, *e.g.*, Chapter 8 Quenching of Fluorescence, pg. 237, col. 1, ¶2 in Principles of Fluorescence Spectroscopy (Ed. Joseph R. Lakowicz, Kluwer

Academic/Plenum Publisher, 2nd Ed.1999), hereafter the "Lakowicz reference.". The Schmidt patent teaches the close placement of the donor and quencher molecules of all disclosed peptides. For example, the Schmidt patent discloses two 17 amino acid substrates with a BoNT/A cleavage site, two 35 amino acid substrates with a BoNT/B cleavage site, and three 39 amino acid substrates containing BoNT/ D and BoNT/F cleavage sites, see, *e.g.*, col. 5, line 20 through col. 6, line 53. However, despite the large peptide sizes, the distance of the donor from the quencher for each substrate is between two and five amino acids.

Second, the close proximity requirement discussed above necessitates that the donor and quencher be located within the amino acid region 191 to 202 of SEQ ID NO: 2. Because this cleavage site region interacts with the catalytic site of the toxin, extensive mutagenesis work was conducted in order to identify which amino acids could be altered and still retain the ability to be cleaved by the toxin. For example, amino acid substitutions surrounding the BoNT/A cleavage site were tested using at least 56 different peptides, see, *e.g.*, James J. Schmidt and Karen A. Bostian, *Assays for the Proteolytic Activities of Serotype A from Clostridium botulinum*, U.S. Patent 5,965,699 (Oct. 12, 1999), hereafter the '699 patent. Substitution of an original amino acid with a different amino acid or an amino acid analog within the amino acid region 191 to 202 of SEQ ID NO: 2 more time than not resulted in an ineffective substrate because the resulting peptide substrate was inefficiently cleavage. This work revealed that only certain amino acids positions within the amino acid region 191 to 202 of SEQ ID NO: 2 could be altered, see *e.g.*, col. 5 through col. 11 and Table I of the '699 patent; pg. 44-45 and Table 3 of the present specification. This work lead to the two BoNT/A substrates disclosed in the Schmidt patent and to the conclusion that modifications to the disclosed substrates are not straightforward because of complex and stringent limitations radiating quenching places on the location of the donor and quencher moieties, see, *e.g.*, col. 7, lines 1-12. Nowhere does the Schmidt patent teach, suggest or motivate one skilled in the art to place a donor or quencher outside of the amino acid region 191 to 202 of SEQ ID NO: 2.

The Holskin reference is silent regarding the positioning either a donor fluorophore or quencher outside of the amino acid segment 191 to 202 of SEQ ID NO: 2. On the contrary,

the teachings of the Holskin reference indicates that both the donor fluorophore and the quencher must be located within the amino acid region 191 to 202 of SEQ ID NO: 2. For example, the Holskin reference teaches that separation of the donor fluorophore EDANS and the quencher DABCYL by more than eight residues results in a dramatic loss of quenching, see, *e.g.*, pg. 154, col. 1, ¶ 1, lines 5-21. In conjunction with the teaching of the Schmidt patent that the placement of the donor and quencher can only be located at certain positions, see above, the Holskin reference would also direct the placement of both EDANS and DABCYL within amino acid region 191 to 202 of SEQ ID NO: 2. Therefore, the Holskin reference does not provide any teaching, suggestion or motivation for one skilled in the art to act contrary to the teachings of the Schmidt patent.

Thus, the Schmidt patent teaches that the donor and quencher of type I substrates can only be separated by 2-5 amino acids and that the placement of the donor and quencher can only be located at certain positions within the amino acid region 191 to 202 of SEQ ID NO: 2. Therefore, the Applicants respectfully submit that a *prima facie* case of obviousness cannot be made because the Schmidt patent teaches away from a BoNT/A substrate as presently claimed because this would result in the placement of a donor fluorophore or acceptor outside the amino acid region 191 to 202 of SEQ ID NO: 2.

Schmidt type II substrates

The type II peptide substrates disclosed in the Schmidt patent, of which the peptides SEQ ID NO: 11 and SEQ ID NO: 12 belong, are used in a fluorescence-release assay using only a single fluorophore. These substrates are 116 residues in length and contain only one fluorophore at position 1, see, *e.g.*, col. 8 lines 1-49. Both of these substrates are intended for immobilization to a solid matrix through reaction of the sulfhydryl groups of the C-terminal cysteine residues at position 116 with maleimide groups, see, *e.g.*, col. 7, lines 15-20. The BoNT/A cleavage site for these substrates is located at positions 106-107, see, *e.g.*, col. 8 lines 1-49. The principle of operation for the fluorescent-release assay using these substrates entails the cleavage of the BoNT/A cleavage site at positions 106-107 by the toxin and the release of the cleavage fragment 1-106 containing the fluorophore from the

solid matrix into a solution. The appearance of fluorescence in the solution indicates the presence of BoNT/A activity, see, *e.g.*, col. 9, lines 7-16.

As discussed above, the Holskin reference disclose a quench release assay that uses a 12 amino acid substrate containing the donor fluorophore EDANS and the quencher DABCYL that are preferably located no more than 8 residues apart, see, *e.g.*, pg. 149, col. 2, ¶ 3 through pg. 150, col. 1, ¶ 2.

In order to make a BoNT/A substrate as suggested by the Examiner, the acceptor would need to be located at residues 108-115 of the Schmidt peptides SEQ ID NO: 11 and SEQ ID NO: 12 because the acceptor must to be placed on the opposite side of the 106-107 cleavage site as the donor fluorophore, see, *e.g.*, col. 5, lines 9-12. Thus this substrate would contain a donor fluorophore at position 1 and an acceptor at position 108-115. This arrangement is expressly taught away by both the Schmidt patent and Holskin reference, because, as discussed above, both references teach that the donor and quencher can only be separated a short distance. Therefore, the Applicants respectfully submit that a *prima facie* case of obviousness cannot be made because the combining the Schmidt patent with the Holskin reference as suggested by the Examiner is taught away from by the cited references.

Schmidt and Holskin combination changes the principle of operation of Schmidt.

According to MPEP §2143.01, if a proposed modification or combination of the prior art would change the principle of operation of the prior art invention being modified, then the teachings of the references are not sufficient to render the claims *prima facie* obvious. The Applicants respectfully submit that it would not be obvious for one of ordinary skill in the art to combine the cited references because combining the Schmidt patent with the Holskin reference would change the principle of operation of the fluorescent-release assay disclosed in the Schmidt patent.

As discussed above, the type II peptides SEQ ID NO: 11 and SEQ ID NO: 12 disclosed in the Schmidt patent are used in a fluorescent-release assay, see, *e.g.*, col. 9, lines 7-16. The principle of operation for the fluorescent-release assay using these substrates entails the

cleavage of the BoNT/A cleavage site at positions 106-107 by the toxin and the release of the cleavage fragment 1-106 containing the fluorophore from the solid matrix into a solution. The appearance of fluorescence in the solution indicates the presence of BoNT/A activity, see, *e.g.*, col. 9, lines 7-16. The principle of operation is the release of the fluorescent fragment from one location to another location. This assay does not rely on any kind of radiating (contact) quenching or non-radiating quenching or non-radiating FRET.

As discussed above, the Holskin reference disclose a quench release assay that uses a 12 amino acid substrate containing the donor fluorophore EDANS and the quencher DABCYL that are preferably located no more than 8 residues apart, see, *e.g.*, pg. 149, col. 2, ¶ 3 through pg. 150, col. 1, ¶ 2.

Adding an acceptor to a Schmidt type II peptide would change the principle of operation from a fluorescent-release assay to a resonance energy transfer assay based on a non-radiating principle of operation. Therefore, the Applicants respectfully submit that a *prima facie* case of obviousness cannot be made because the combining the Schmidt patent with the Holskin reference as suggested by the Examiner would change the principle of operation of the fluorescent-release assay disclosed in the Schmidt patent.

Schmidt and Holskin combination provide no motivation to combine.

According to MPEP §2143.01, the mere fact that references can be combined or modified does not render the resultant combination obvious unless the references also suggests the desirability of the combination. The Applicants respectfully submit that it would not be obvious for one of ordinary skill in the art to combine the cited references because there would be no desirability to make the proposed combination.

As discussed above, the type II peptides SEQ ID NO: 11 and SEQ ID NO: 12 disclosed in the Schmidt patent are BoNT/A substrates containing a single fluorophore used in a fluorescent-release assay. In this assay, an uncleaved substrate is linked to a solid support matrix. Upon cleavage by the toxin, the fragment containing the fluorophore is released from the matrix and collected in a flow through solution, see, *e.g.*, col. 9, lines 7-16. The BoNT/A substrate, whether uncleaved or cleaved, is always fluorescing. Toxin activity is

measured by where the fluorescence is located, either on the solid-support matrix (uncleaved) or in the flow-trough solution (cleaved).

As discussed above, the Holskin reference discusses a 12 amino acid substrate containing a donor fluorophore and a quencher used in a quench-release assay. In this assay, fluorescence from donor fluorophore of the uncleaved substrate is quenched by the quencher, see, *e.g.*, pg. 152, col. 1, ¶ 3, Figure 5. Upon cleavage, the donor fluorophore is separated from the quencher, thereby allowing the donor fluorophore to fluoresce. Toxin activity, therefore, is measured by the appearance of fluorescence after cleavage by the toxin.

The Applicant's respectfully submit that there would be no desirability to combined the Schmidt patent and the Holskin reference make a BoNT/A substrate containing a donor fluorophore and an acceptor for use in a fluorescent-release assay because toxin activity is determined by the location of where the fluorescence is present and not whether fluorescents appears (*i.e.*, a quench-release assay). There would be no practical reason to make this proposed substrate because, in a fluorescence-release assay, it is immaterial whether the fluorescence from the bound substrates on the solid matrix can be detected or quenched because neither measurement is indicative of toxin activity. Therefore, the Applicants respectfully submit that a *prima facie* case of obviousness cannot be made because there was no desirability by one skilled in the art to combine the Schmidt patent with the Holskin reference as suggested by the Examiner.

Conclusion

For the reasons stated above, the Applicants respectfully submit that the assertion of obviousness is unsupported by the cited references because the suggested modifications required to produce the presently claimed invention are expressly taught away by the Schmidt patent. Therefore, the Applicants respectfully request withdrawal of the 35 U.S.C. § 103(a) obviousness rejection for Claims 102 and 113-117.

II. Obviousness rejections over Schmidt in view of Holskin and Mahajan

The Examiner has rejected Claims 126-141 as allegedly being obvious under 35 U.S.C. § 103(a) over Schmidt patent in view of the Holskin reference and Nupam P. Mahajan et al., *Novel Mutant Green Fluorescent Protein Protease Substrates Reveal the Activation of Specific Caspases During Apoptosis*, 6(6) Chem. Biol. 401-409 (1999), hereafter the "Mahajan reference."

The Examiner contends that it would have been *prima facie* obvious to a person of ordinary skill in the art at the time the invention was made to combine the teaching of these references and come up with the BoNT/A substrates as presently claimed in Claims 126-141. Specifically, the Examiner argues that it would have been obvious to modify the peptide substrates disclosed in the Schmidt patent or modify the Schmidt patent peptides containing the fluorophore EDANS and quencher DABCYL as modified by the Holskin reference with fluorescent proteins as disclosed in the Mahajan reference. The Applicants respectfully ask for reconsideration under 37 C.F.R. § 1.111.

Schmidt, Holskin and Mahajan combination makes Schmidt unsatisfactory for its intended use.

According to MPEP §2143.01, if proposed modification would render the prior art invention being modified unsatisfactory for its intended purpose, then there is no suggestion or motivation to make the proposed modification. The Applicants respectfully submit that it would not be obvious for one of ordinary skill in the art to combine the cited references because combining the Schmidt patent with the Mahajan reference or the Schmidt patent and the Holskin reference with the Mahajan reference would render the quench-release assay disclosed in the Schmidt patent as inoperable for its intended use because such a combination would prevent the toxin from cleaving the substrate.

The Mahajan reference discloses substrates containing a four amino acid cleavage site flanked by either a BFP-GFP or a CFP-YFP fluorescent protein pair useful for measuring the activity of caspase-1 and caspase-3, see, *e.g.*, pg. 402, col. 2, ¶ 2, lines 1-6. Each of these

fluorescent proteins is approximately 238 amino acids in length and approximately 27 kDa, see, *e.g.*, pg. 402, col. 1, ¶ 1, lines 1-2.

The Schmidt patent discloses peptide substrates useful for quench release assays. As discussed above, the Schmidt patent teaches that these peptides require the donor and quencher be separated by no more than 2 to 5 amino acids and that this placement must be at certain positions within the amino acid region 191 to 202 of SEQ ID NO: 2.

The placement of an approximately 238 amino acid fluorescent protein taught by Mahajan reference into the amino acid region 191 to 202 of SEQ ID NO: 2 in the positions identified in the Schmidt patent, as suggested by the Examiner, would result in an inoperable peptide substrate. First, work has shown that a BoNT/A peptide substrate requires a length of 16 amino acid peptide before appreciable cleavage can be seen, see, *e.g.*, the '699 patent at col. 14, lines 10-49. Substitution of a fluorescent protein into the sites identified by the Schmidt patent would divide the 16 amino acid peptide region necessary for cleavage into two inoperable fragments, thereby making such a substrate unsuitable for the activity assay disclosed in Schmidt. Second, placement of the fluorescent proteins in a manner required to maintain continuity of the 16 amino acid peptide necessary for functionality is contrary to the radiating quenching teachings of Schmidt, discussed above, which require that the donor and quencher be separated by 2-5 amino acids.

The Hoskin reference also teaches the positioning of EDANS and DABCYL within the amino acid region 191 to 202 of SEQ ID NO: 2, as discussed above. Thus this reference cannot cure the deficient teachings of the Schmidt patent. Therefore, the Holskin reference does not provide any teaching, suggestion or motivation for one skilled in the art to act contrary to the teachings of the Schmidt patent.

Thus, adding donor and acceptor fluorescent proteins to a Schmidt peptide would make these peptides inoperable for their intended use in quench-release assay as disclosed in the Schmidt patent. Therefore, the Applicants respectfully submit that a *prima facie* case of obviousness cannot be made because combining the Schmidt patent and the Holskin

reference with the Mahajan reference as suggested by the Examiner would result in an inoperable substrate.

Schmidt, Holskin and Mahajan combination changes the principle of operation of Schmidt.

According to MPEP §2143.01, if a proposed modification or combination of the prior art would change the principle of operation of the prior art invention being modified, then the teachings of the references are not sufficient to render the claims *prima facie* obvious. The Applicants respectfully submit that it would not be obvious for one of ordinary skill in the art to combine the cited references because combining the Schmidt patent with the Mahajan reference or the Schmidt patent and the Holskin reference with the Mahajan reference would change the principle of operation of the quench-release assay disclosed in the Schmidt patent.

The Schmidt patent relies on a quenching assay where the fluorescence of a donor is prevented by a quencher, see, *e.g.*, col. 4, lines 14-17; col. 5, lines 9-15; and col. 6, lines 54-59. Enzyme activity is measured as an increase in fluorescence because cleavage of the toxin substrate results in the separation of the donor from the quencher. Separation leads to the unquenching of the donor and a corresponding increase in donor in fluorescence, see, *e.g.*, col. 5, lines 8-15; and col. 6, lines 54-62. The quenching-based assays disclosed in the Schmidt patent rely on the principle of operation called radiating (contact) quenching. One of the hallmarks of the operative principle of radiating quenching is the close proximity of the donor and quencher, a distance calculated by the Stern-Volmer equation, see, *e.g.*, Chapter 8 Quenching of Fluorescence, pg. 239, col. 2, Sections 8.2 and 8.3 in the Lakowicz reference. This is because in radiating quenching, the quenching effect occurs by the physical contact of the electron orbits of the donor and quencher molecules, see, *e.g.*, Chapter 8 Quenching of Fluorescence, pg. 237, col. 1, ¶2 in the Lakowicz reference.

The enzyme activity assay disclosed in the Mahajan reference relies on the excitation of an acceptor fluorescent protein using the energy transferred from a fluorescent donor protein, a principle of operation called fluorescence resonance energy transfer (FRET). Enzyme activity is measured as a decrease in energy transfer because cleavage of the caspase

substrate leads to the separation of the two fluorescent proteins. This separation leads to a loss of energy transfer from the donor to the acceptor and a corresponding loss of acceptor fluorescence, see, *e.g.*, pg. 403, col. 1, ¶ 1, lines 1-16; pg. 403, col. 2, ¶ 1, lines 1-7; and pg. 406, col. 1, ¶ 2, lines 1-10. FRET is a non-radiating-based principle of operation. In this principle, energy is transferred from one molecule to another by intermolecular long-range dipole-dipole coupling, see, *e.g.*, Chapter 13 Energy Transfer, pg. 367, col. 1, ¶1 in the Lakowicz reference. In this principle of operation, energy transfer depends on the extent of spectral overlap of the donor emissions spectrum and the acceptors absorption spectrum, and not on the physical contact of electron orbits of the molecules. The optimal spectral overlap distance is calculated using the Förster equation and not the Stern-Volmer equation. Thus, the Applicant's respectfully disagree with the Examiner's statement that FRET assays are also known as quenched-signal assays. These assays operate on two distinct principles of operation.

The Holskin reference also discloses a quench-release assay and, therefore, can not make up for any deficiency of the teachings of the Majahan reference and Schmidt patent. This reference describes a 12 amino acid substrate containing the donor fluorophore EDANS and the quencher DABCYL, see, *e.g.*, pg. 149, col. 2, ¶ 3 through pg. 150, col. 1, ¶ 2. Like the Schmidt patent, enzyme activity is measured as an increase in fluorescence because cleavage of the toxin substrate results in the separation of the donor from the quencher and a corresponding increase in donor in fluorescence, see, *e.g.*, pg. 152, col. 1, ¶ 3, Figure 5. Therefore, the Holskin reference does not provide any teaching, suggestion or motivation for one skilled in the art to overcome the deficient teaching of the Schmidt patent.

Thus, adding donor and acceptor fluorescent proteins to a Schmidt peptide would change the principle of operation from a quench-release assay based on a radiating principle of operation to a resonance energy transfer assay based on a non-radiating principle of operation. Therefore, the Applicants respectfully submit that a *prima facie* case of obviousness cannot be made because the combining the Mahajan reference with Schmidt patent as suggested by the Examiner would change the principle of operation of the quench release assay disclosed in the Schmidt patent.

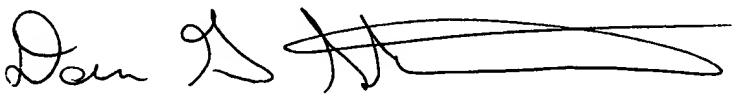
Conclusion

For the reasons stated above, the Applicants respectfully submit that the assertion of obviousness is unsupported by the cited references because the suggested modifications required to produce the presently claimed invention would result in an inoperable quench release assay as disclosed in the Schmidt patent and would change the principle of operation of the quench release assay as taught in the Schmidt patent. Therefore, the Applicants respectfully request withdrawal of the 35 U.S.C. § 103(a) obviousness rejection for Claims 126-141.

CONCLUSION

For the above reasons the Applicants respectfully submit that the claims are in condition for allowance, and the Applicants respectfully urge the Examiner to issue a Notice to that effect. Should the Examiner have any questions, he is invited to call the undersigned agent. Please use Deposit Account 01-0885 for the payment of any extension of time fees under 37 C.F.R. § 1.136 or any other fees due in connection with the current response.

Respectfully submitted,



Dean G. Stathakis, Ph.D.
Registration No. 54,465
Agent of Record

ALLERGAN
LEGAL DEPARTMENT
2525 Dupont Drive
Irvine, California 92612-1599
Tel: 714/246-6521
Fax: 714/246-4249